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The most common research species is the laboratory rodent. In physiological research, rodents often undergo procedures that require the use of anesthesia. For such small animals, anesthetic is typically administered by systemic injection. Advantages of this technique include ease of administration and low cost. The main disadvantage of this technique is the lack of control over anesthesia depth. As such, toxicity and death are a hazard, which confounds experiments and just as importantly, the care and welfare of animals used in research. Inhalational anesthesia is an alternative method that because of its enhanced control is associated with few adverse side effects. While small animal inhalational anesthesia machines are commercially available, the prohibitive cost of these devices often discourages their application in research. The conversion of a conventional anesthesia system for large animals to one that can support inhalation anesthesia in small animals would conserve research resources, improve small animal research quality and enhance animal care and welfare. This report describes a simple, safe, effective, and efficient technique to make such a conversion.

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USARIEM TECHNICAL REPORT T04-04

CONVERSION OF A LARGE ANIMAL INHALATIONAL ANESTHESIA MACHINE TO ONE FOR SMALL ANIMAL USE

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DISCLAIMER STATEMENT

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals" as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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BACKGROUND

The most common research species is the laboratory rodent. In physiological research, rodents often undergo procedures that require the use of anesthesia. For such small animals, anesthetic is typically administered by systemic injection. Advantages of this technique include ease of administration and low cost. The main disadvantage of this technique is the lack of control over anesthesia depth. As such, toxicity and death are a hazard, which confounds experiments and just as importantly, the care and welfare of animals used in research. Inhalational anesthesia is an alternative method that because of its enhanced control is associated with few adverse side effects. While small animal inhalational anesthesia machines are commercially available, the prohibitive cost of these devices often discourages their application in research. The conversion of a conventional anesthesia system for large animals to one that can support inhalation anesthesia in small animals would conserve research resources, improve small animal research quality and enhance animal care and welfare. This report describes a simple, safe, effective, and efficient technique to make such a conversion.

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MAJ William Fall is recognized for his expert instruction in anesthesia machine maintenance and operation.

EXECUTIVE SUMMARY

The use of injectable anesthesia in animal research practices has the disadvantage of uncontrollable anesthesia depth, which leads to toxicity and at times death. This adversely influences research and, animal care and welfare. Injectable anesthesia is generally employed in small research animals, since body mass issues prevent the use of conventional inhalation anesthesia machines, while specialized small animal devices are too costly. To support small animal research in mice and rats, a conventional inhalation anesthesia machine, suitable only for large animals, was converted such that it could also support small animal anesthesia. This conversion was rapidly and easily achieved employing inexpensive materials generally found in most laboratories. Multiple animals could be simultaneously anesthetized, such that surgical procedures in large groups of animals could be completed in less time. Animals rapidly recovered from anesthesia to become ambulatory. No anesthesia-related deaths were recorded. This modification employed effective strategies to limit anesthetic gas contamination of the outside environment, such that exposures to personnel were within acceptable limits. Such a conversion of inhalation anesthesia equipment provided an effective, efficient and safe alternative to the use of injectable anesthetics in small research animals. Its application supported conservation of research resources, reduced anesthesia-related confounding factors on research quality, and improved animal care and welfare.

INTRODUCTION

Anesthesia machines are generally defined as either non-rebreathing (open) or rebreathing (semi-closed) systems. Rebreathing systems require the patient or animal to have an inspiratory/expiratory capacity that can overcome resistance within the anesthesia machine circuitry to sustain airflow. Due to a small tidal volume and rapid respiratory rate, animals that weigh less than 7 kg cannot overcome the resistance associated with the mechanical airflow valves of conventional rebreathing systems. As such, small animals require a non-rebreathing design. Research laboratories employing both small and large animals necessitate the need for both types of anesthesia delivery systems, which burdens limited resources. The present report describes a technique to convert a conventional rebreathing, inhalation anesthesia machine to a non-rebreathing system. Employing this conversion technique, a single anesthesia machine could support rebreathing and non-rebreathing applications. Thus, small research animals, such as rodents could avoid injectable anesthesia procedures which, because these procedures provide less control over anesthesia depth, are more often associated with toxicity and death. As a result of this conversion, research resources were conserved, research quality was improved, and animal care and welfare were enhanced.

METHODS

A Drager Narkovet Deluxe (North American Drager, Telford, PA) gas anesthesia machine was converted from large to small animal use. The following materials were required in this conversion:

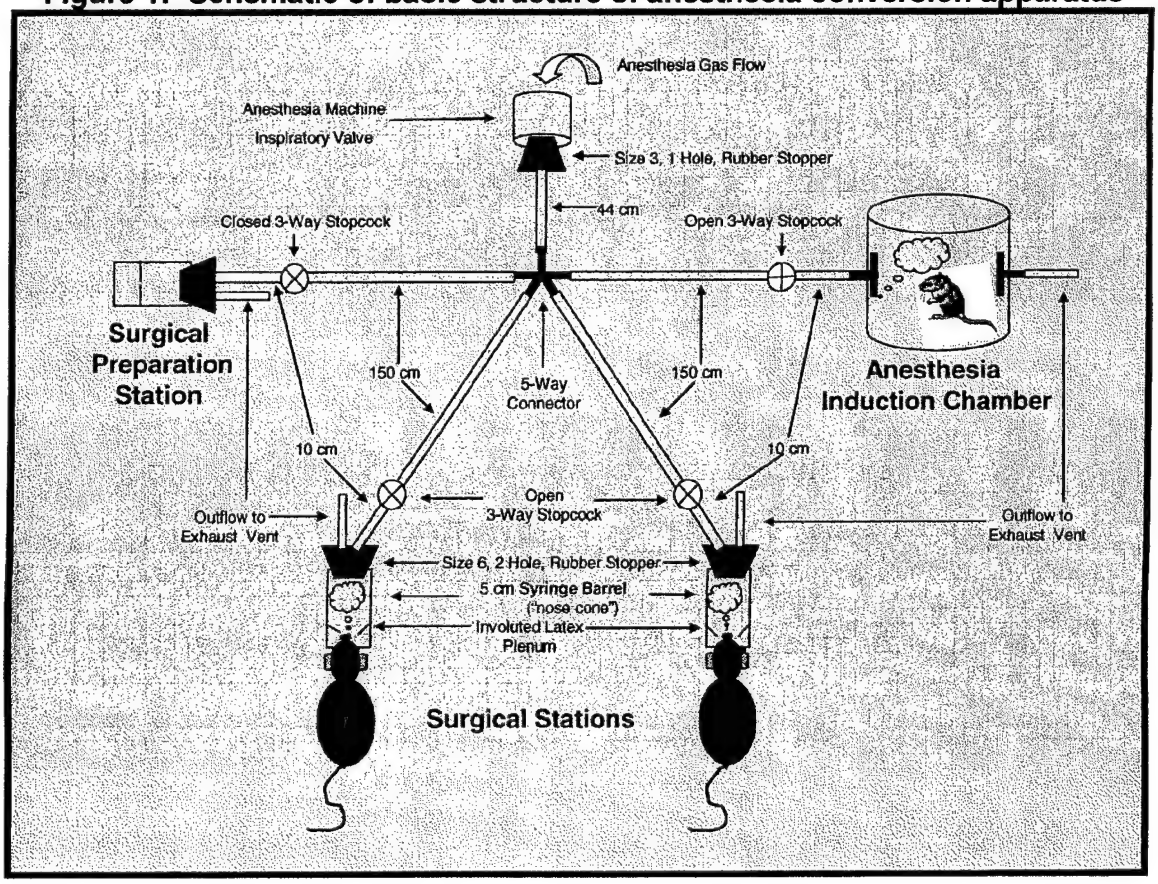
60 cc syringes (N=3)
Tygon tubing (ID 1/8" x OD 1/4") 10 cm (N=4), 44 cm (N=1), 150 cm (N=8), and 400 cm (N=1) lengths.
3-way premium stopcocks (N=4)
Two-hole rubber stoppers, size 6, hole size 6 mm (N=3)
One-hole rubber stopper, size 3, hole size 6 mm (N=1)
Plastic connectors:
    Type T: ID 4 mm x OD 5 mm (N=4)
    Type 5-way: ID 4 mm x OD 5 mm (N=2)
Plastic bowl (Tupperware®, size 7, 705 ml) (N=1)
Plastic bowl (Tupperware®, size 3, 1.4 L) (N=1)
Large latex surgical gloves (N=3)
Tape
Silicone adhesive
Drill bit, 4 mm

Inflow Tubing Assembly (Fig 1): One end of a 44 cm length of Tygon tubing was inserted into the size 3, one-hole, rubber stopper that was secured in the inspiratory

valve of the conventional anesthesia machine. The other end of the tubing was placed into one branch of a 5-port plastic connector. To each of the four remaining branches of the 5-port connector, a 150 cm length piece of Tygon tubing was applied. To the opposite ends of these tubing pieces, 3-way stopcocks were connected. Extending from each of these four stopcocks were 10 cm lengths of Tygon tubing, one connected to the T connector of the induction chamber while the other three were attached to the size 6, two-hole rubber stopper found in each of the syringe barrel assemblies.

Anesthesia Induction Chamber Assembly (Fig 1): A 4 mm drill bit was used to drill two holes into each plastic bowl, directly across from one another. These holes served as inflow and outflow ports. One T-type plastic connector was inserted into each hole and secured with silicone adhesive to prevent efflux of gas anesthetic to the surrounding air. The small bowl was used as the anesthesia induction chamber for mice, while the larger bowl was employed for rats (maximum body weight ~350 g).

Figure 1: Schematic of basic structure of anesthesia conversion apparatus



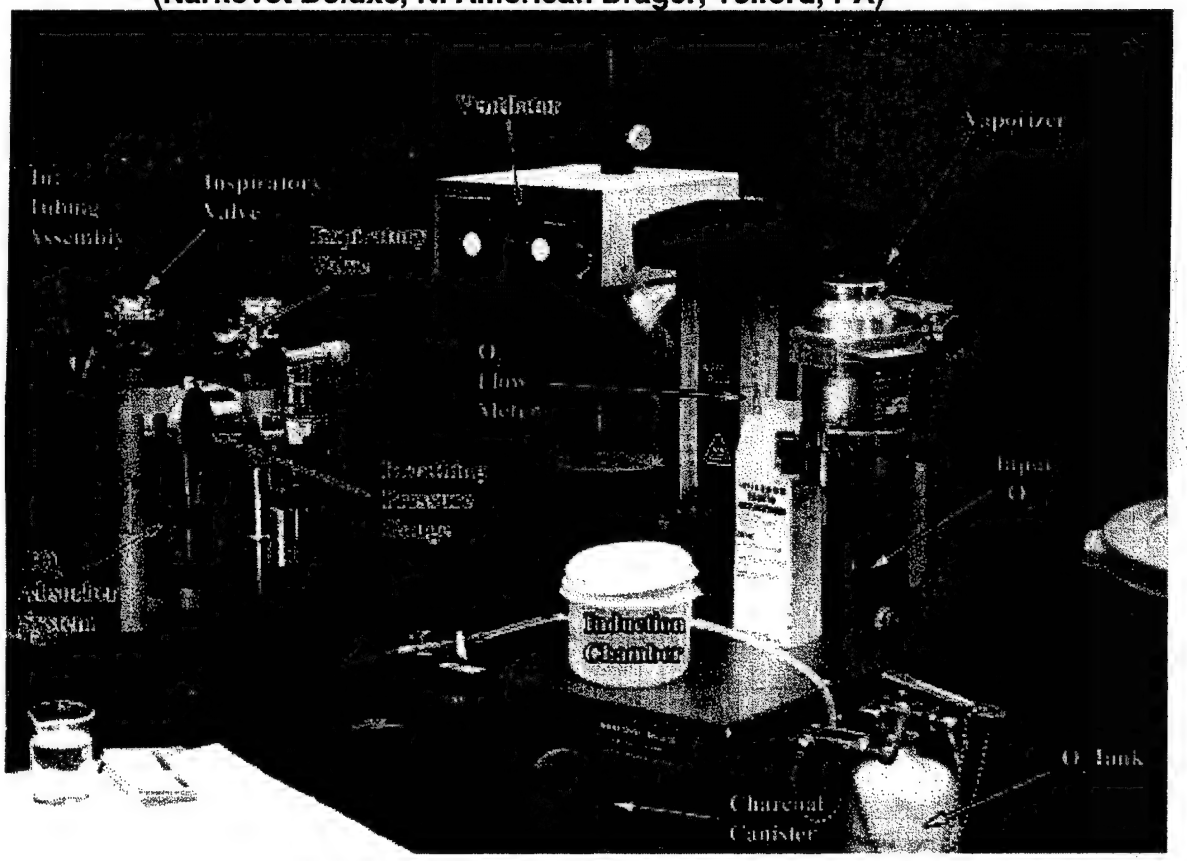
Syringe Barrel Assembly (Fig 1): The plunger from each 60 cc syringe was discarded and the syringe barrels cut to a length of 5 cm. A size 6, two-hole, rubber stopper was placed in one end of each syringe. To reduce syringe barrel aperture, such that anesthetic gas efflux was minimized, a 3 cm length of latex from the middle finger of a large surgical glove was placed over the open end of each syringe barrel. It was

then secured with tape on the outside and the latex material involuted into the syringe barrel to form a plenum.

Outflow Tubing Assembly (Fig 1): A 150 cm length of Tygon tubing was attached to the outflow port of the induction chamber and the size 6, two-hole, rubber stopper of each syringe barrel. The free ends of these four tubing pieces were connected to a second 5-way plastic connector that was attached to a single 400 cm length of tubing that led into an exhaust vent to remove expired CO_2 from the animal, and excess anesthetic gas.

Machine Operation: Positive pressure provided by the flow of oxygen (O_2; 0.6 L/min) moved anesthetic gas through an open loop system, using the conversion apparatus (Fig 1) attached to a conventional anesthesia machine (Fig 2). O_2 pressure was regulated by a flow meter to the vaporizer where the liquid isoflurane (Abbott, Labs. N. Chicago, IL) was vaporized into an O_2/anesthetic gas mixture (2.5%). This mixture passed through the inspiratory valve where it was distributed via the 5-port connector to the various stations as depicted in Figure 1. To scavenge refluxing anesthetic gas that resulted due to system resistance, a charcoal canister (F/Air; Bickford, Inc.; Wales Center, NY) was connected to the inspiratory valve.

Figure 2: Conventional, semi-closed, anesthesia machine for large animals (Narkovet Deluxe, N. American Drager, Telford, PA)



Air Quality Testing: Real-time monitoring of waste isoflurane emissions in the surgical environment was performed using an AIM 3250 detector (Allanco Iolite Systems, LTD, Canada). This instrument was calibrated with calibration gas an hour before sampling and allowed to equilibrate in a clean atmosphere for 10 minutes. Air sampling zones were as follows: (1) floor level, (2) 8 feet above the floor, (3) immediately in front of the syringe barrel, (4) surgical personnel working area, and (5) inspiratory inflow valve (positive control). Airflow in the surgical suite was 10.2 exchanges/h.

RESULTS

Figure 1 illustrates the basic features of the conversion apparatus employed to convert a conventional rebreathing (semi-closed) anesthesia machine to a non-rebreathing (open) system capable of supporting small research animals. As illustrated, the conversion apparatus was connected to the anesthesia machine via a rubber stopper that interfaced with the anesthesia machine inspiratory valve. Gas flow from the machine was diverted to four stations via tubing connected with the 5-port connector. The tubing, which extended from 4 branches of the 5-port connecter to the various stations (inhalation induction chamber, surgical preparation and surgical stations), were of the same length. These pieces of tubing were associated with 3-way stopcocks that permitted control of gas flow to each station. Following an equilibration period (~30 min) to saturate the system, a mouse (28±3 g) placed in the induction chamber was anesthetized within 3±1 min. It remained anesthetized as it progressed from surgical preparation to the surgical station by placing its nose within the plenum of the syringe barrel formed by the involuted latex. This allowed exposure to a small eddy of gas at the end of the syringe barrel.

Figure 2 shows the conventional Narkovet Deluxe anesthesia machine that was modified. A mouse inhalation induction chamber with a 3-way stopcock, charcoal canister for waste gas scavenging, and the inspiratory valve with tubing through which the O_2/anesthetic gas mixture was delivered to the conversion apparatus are shown. Also shown is the auxiliary O_2 gas cylinder that can be employed for procedures conducted outside the surgical suite. As a result of its conversion, several components of the anesthesia machine were not engaged during non-rebreathing operation. These included the ventilator, absorber system for exhaled CO_2, breathing pressure gauge, and expiratory valve.

Figure 3 shows the surgical preparation station. Following anesthesia induction, as described above, anesthesia was sustained by placing the animal's nose at the end of the syringe barrel assembly. Exposure to the eddy of gas anesthetic effectively sustained anesthesia to allow clipping of animal fur and sterilization of the shaved surface with iodine (10% Povadyne®; Chaston, Inc., Dayville, CT).

Figure 4 shows the surgical stations with the syringe barrel assemblies. The clear inflow tubing to each syringe barrel was equipped with a 3-way stopcock for control of anesthesia delivery. The opaque outflow tubing allowed flow of waste gas anesthetic and exhaled CO_2 from the animal to an exhaust system. Anesthesia was

sustained at this station for as long as 30±10 min to complete a laparotomy. The capacity to perform simultaneous surgeries at multiple surgical stations supported completion of procedures on large groups of animals in less time. Within 1±0.5 or 4±2 min of completion of surgery, mice or rats, respectively regained consciousness and became ambulatory. In over 200 mouse and rat laparotomies, no observable adverse physiological effects or mortalities were related to the induction of anesthesia.

Figure 3: Surgical preparation station for fur clipping and iodine sterilization

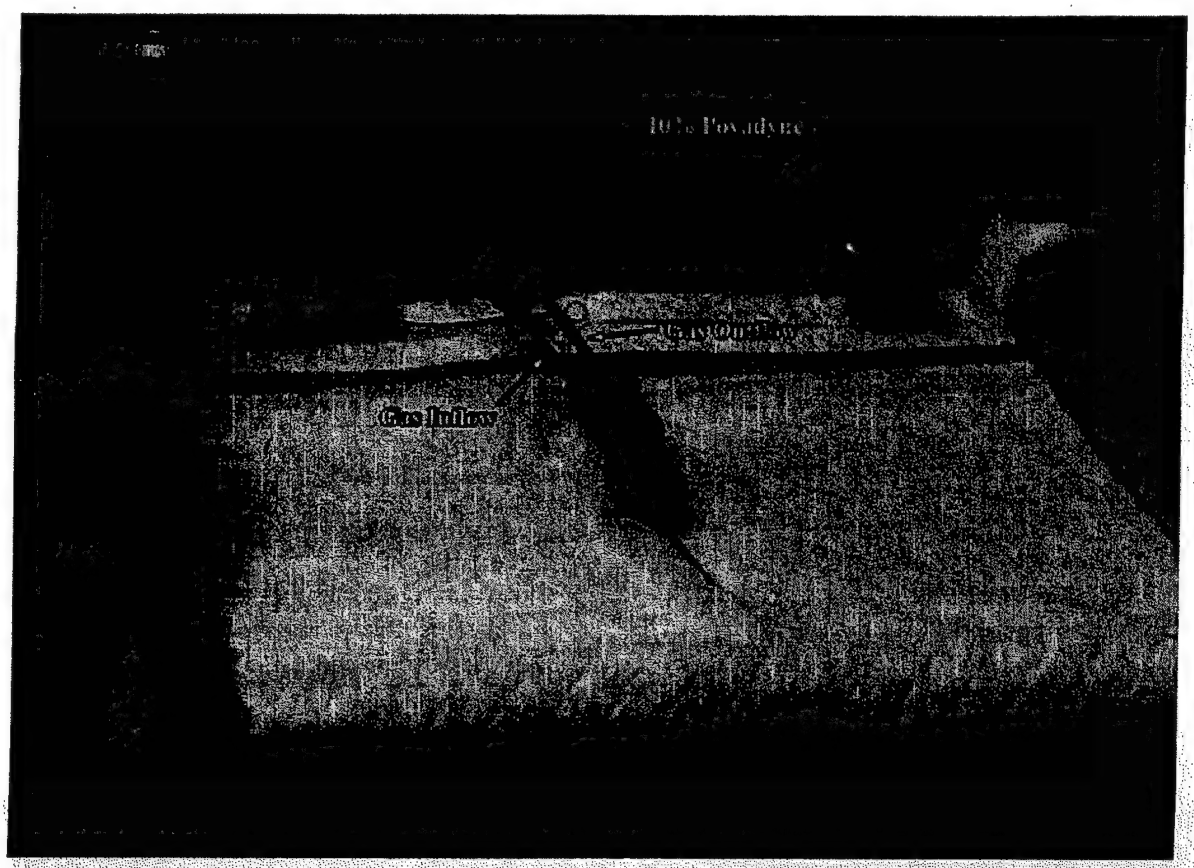


Figure 5 shows the capacity of the syringe barrel design to support anesthetic delivery to a rat, a small research animal, but with a larger body mass (10X) than a mouse. In spite of the differences between a mouse and rat in regards to the degree of obstruction imposed at the end of the syringe barrel, the involuted latex plenum functioned to reduce gas efflux to the outside atmosphere.

Air Quality Testing: Isoflurane concentration was detected at 40,000 ppm at the inspiratory valve of the anesthesia machine (positive control). The air at the opening at each syringe barrel measured 30-40 ppm, whereas all readings within the working area of surgical personnel ranged from 2-4 ppm.

Figure 4: Dual surgical stations to support simultaneous surgical procedures

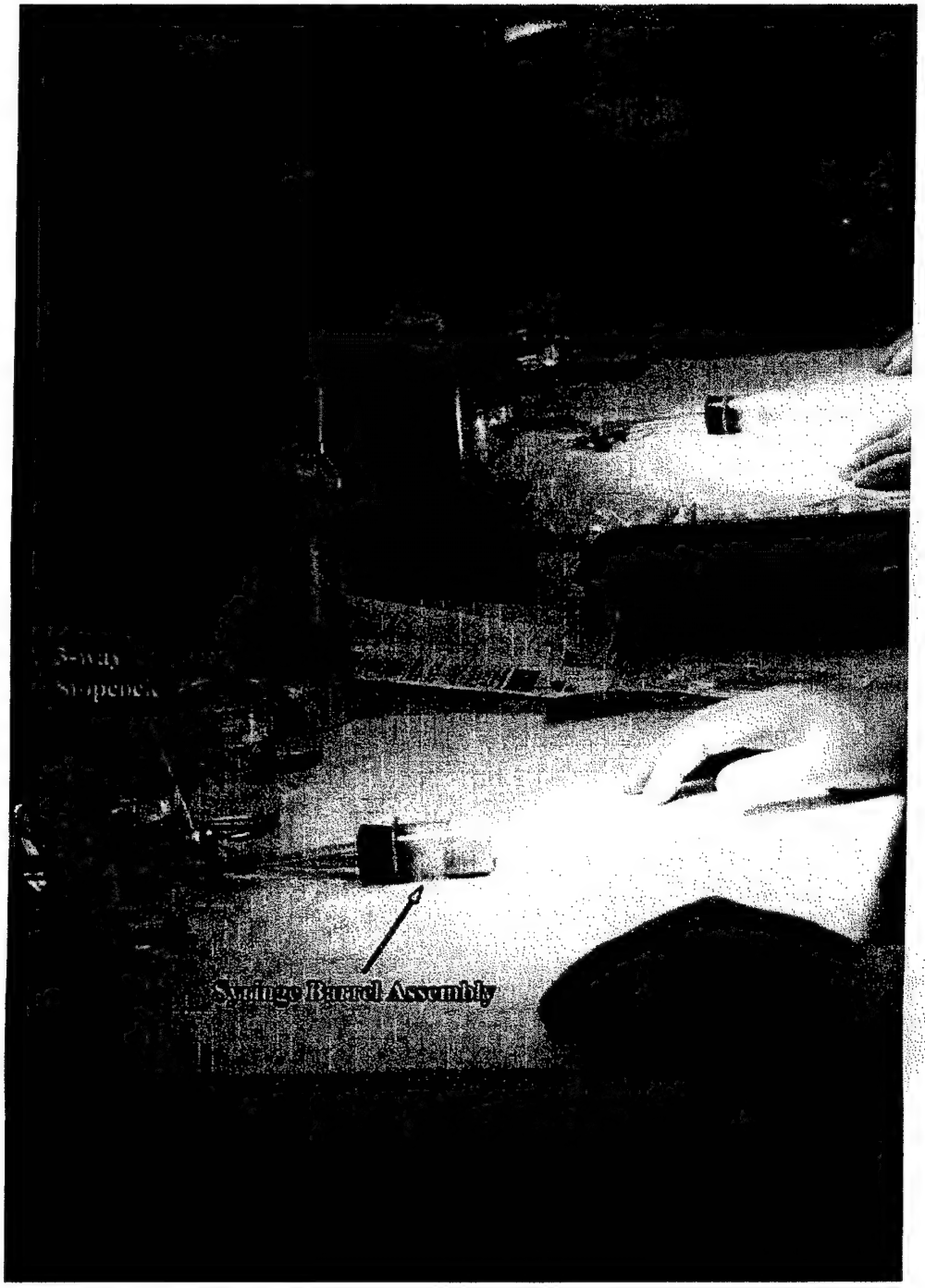
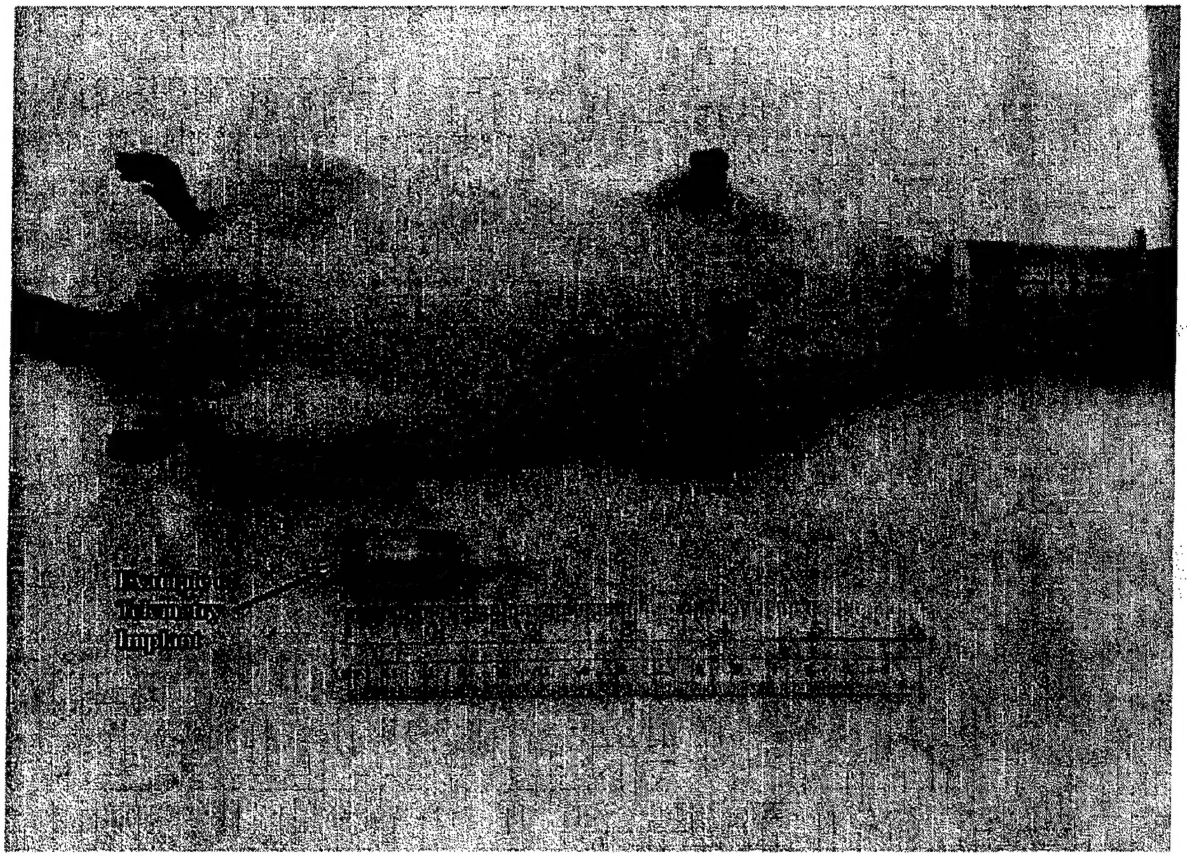


Figure 5: Illustration of the capacity of the syringe barrel design to accommodate a rat that is of larger mass (10x) than a mouse



DISCUSSION

Injectable anesthetics are commonly used for surgical procedures performed on rodents, despite inefficient control of anesthetic depth, the potential of a toxic exposure and prolonged recovery periods. Several hours may be required for effective metabolism of injectable anesthetics, which can lead to hypothermia, respiratory depression and perhaps death. As such, research quality and, animal care and welfare suffer. To avoid these adversities, inhalation anesthesia is preferable, since small rodents rapidly recover and experience few side effects. Inhalation anesthesia is not generally employed, since equipment designed specifically for small rodents (i.e., 20-300 g animals) is rather costly. However, a conventional, semi-closed, rebreathing, inhalational anesthesia machine for large animals was effectively converted to an open, non-rebreathing device suitable for use with small animals (mice and rats).

This conversion was achieved with inexpensive materials found in most laboratories and, made for an easy and rapid conversion process. To ensure a similar gas flow, a standard tubing length was employed that connected the anesthesia

machine to the various stations of the conversion apparatus (Fig 1). Gas flow to these areas could be further controlled by adjustment of the 3-way stopcock. These features supported an even anesthetic delivery throughout the converted apparatus. Regardless of when mice or rats were anesthetized, the plenum formed by the involuted latex at the end of the syringe barrow assembly, in conjunction with outflow tubing connected to an exhaust vent, reduced anesthetic gas release to the outside atmosphere. This conversion design permitted completion of surgical procedures on large groups of animals in less time, since it provided multiple surgical stations. Moreover, inhalation anesthesia for small animals distant from the surgical suite was supportable, because an auxiliary O_2 gas cylinder is associated with the conventional system (Fig. 2). Since many conventional systems employ a similar operational format, this conversion technique should be applicable to equipment available from a number of vendors.

The ease of induction of inhalation anesthesia and the rapid recovery of mice or rats employing an anesthesia machine converted to an open, non-rebreathing system was a testament to the success of the conversion process. Moreover, the absence of adverse reactions in the animals demonstrated that the capacity to use inhalation anesthesia through this conversion technique should enhance research quality and, animal welfare and care.

While inhalation anesthesia greatly benefits the small animal, the conversion to an open anesthesia system has consequences that could impact the health of personnel, as a result of exposure to anesthetic gas. Odor is commonly described as a potential detection method for isoflurane air contamination. Though the olfactory threshold for isoflurane is not clearly defined, that of the closely related halogenated gas halothane is 50 ppm (3, 5). Thus, olfactory detection might result in exposure to rather high levels of isoflurane. The atmospheric level of waste gas depends on the anesthetic concentration (1), carrier gas flow (2), and air turnover in the surgical theater (4). OSHA has not established a permissible exposure limit for halogenated anesthetic agents, such as isoflurane and halothane. However, the American Conference of Government Industrial Hygienists sets a threshold limit value for isoflurane of 75 ppm over an 8 h work day, while the National Institute for Occupational Safety and Health recommends a limit of 2 ppm over 60 min. Under the conditions described in this report, the outside atmospheric contamination for isoflurane within the working area of surgical personnel was within this range. This indicated personnel were not excessively exposed to anesthetic gas as a result of the conversion of the conventional anesthesia machine to an open, non-rebreathing system.

CONCLUSIONS

A conventional, semi-closed, rebreathing, anesthesia machine was easily converted to an open, non-rebreathing system suitable for use with small research animals. Materials employed in its modification were readily available and inexpensive. The simplicity of the method permitted this conversion in a short period of time. The converted machine induced a state of anesthesia from which animals rapidly recovered in the absence of severe adverse effects. It supported multiple surgical stations, was

portable, and controlled anesthetic gas contamination of the outside atmosphere within acceptable limits. Since a single machine could now support rebreathing or non-rebreathing applications, this conversion technique conserved research resources. Moreover, the capacity to employ inhalation anesthesia relieved small animals from exposure to the vagaries associated with injectable anesthetic, improved research quality, and promoted animal care and welfare.

RECOMMENDATIONS

Since inhalation compared to injectable anesthesia is less toxic and avoids adverse physiological reactions to include mortality, it is recommended the conversion technique, as described above, be employed such that conventional, rebreathing anesthesia machines could be used to support non-rebreathing anesthesia for small research animals.

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